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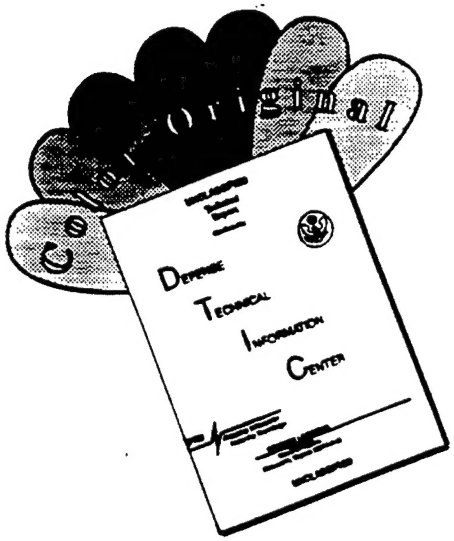

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During the previous year we have investigated the effects of interleukin-1 (IL-1) and interleukin-6 (IL-6) on the density and function of beta to 2 adrenergic receptor (AR) protein and steady state mRNA levels in human lymphoblastoid cell lines. We established a DNA-excess solution hybridization assay using M13-beta-2AR (111) template DNA. This assay helped us detect small changes in the message for beta-2AR. We treated Epstein-Barr (EB) virus transformed human B lymphoblastoid cells and an antibody-secreting lymphoblastoid cell line (IM9) with IL-1 and IL-6. Treatment of the cells with the cytokines caused a marked decrease in the density of beta-2AR but failed to change the affinity of the receptors for the ligand. In contrast, treatment of B cells with both cytokines resulted in increase in the beta-2AR message in both cell lines. To investigate the discrepancy between the effect of lymphokines on receptor protein and its message, we conducted a transcription experiment by using nuclear run-off assays in which we demonstrated that lymphokines increase the stability of the mRNA for beta-2AR. These results demonstrate that lymphokines may alter the expression of stress receptors in human lymphocytes. We conclude that stress receptors interact at the molecular level with elements of the immune system.

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INTRODUCTION

The presence of ß2-adrenergic receptors (ß2AR) on the surface of lymphocytes makes them susceptible to sympathetic stimulation. ß2AR agonist stimulation inhibits lymphocyte proliferation, mitogen induced secretion of IL-2 and expression of IL-2 receptors. The density of lymphocyte $ß_2$AR is influenced by a variety of physiological and pathophysiological conditions, including stress. There is evidence that shock-induced immune suppression is at least partially mediated by adrenal hormones and peripheral ßAR. Interleukins alter the ßAR responsiveness in lymphocytes as well as in pituitary cells suggesting that they may have an important functional role in the integrated regulatory response to environmental stimuli.

In the present study, we have investigated the effects of IL-1 and IL-6 on the density and function of $ß_2$AR protein and steady state mRNA levels in human lymphoblastoid cell lines.

METHODS

Cells and treatment. Epstein-Barr virus (EBV)-transformed normal human B-lymphocytes and an antibody secreting lymphoblastoid cell line (IM-9, ATCC) were treated with IL-1a and IL-6 (30 IU/ml) for 24 hrs, unless otherwise indicated.

Receptor binding assay. The affinity and density of ßAR were assessed by receptor binding assay using ^{125}I-iodocyanopindolol as radioligand. The saturation isoterms were evaluated using the LIGAND computer program.

DNA-excess solution hybridization assay. In order to selectively measure the amount of the ß2AR mRNA in total RNA preparations from lymphocytes, a 111 nucleotide long single stranded cDNA probe specific for the mRNA of the human $ß_2$AR was synthesized and used in a DNA-excess solution hybridization assay as previously described (Szentendrei et al., J. Cell Physiol. 152:478, 1992). The assay is sensitive enough to reproducibly detect minor changes in the steady state level of the ß2AR mRNA, which proved to be 0.2-0.3 amol mRNA/mg total RNA in the lymphocyte cell lines studied. The sensitivity and linearity of the assay is illustrated on the standard curve which is used to calculate the unknown amount of mRNA (Fig. 1.)

cAMP accumulation. The cAMP accumulation was assayed in intact lymphocytes following 10 min stimulation with 10 µM isoproterenol using a commercial cAMP assay kit (Amersham).

Figure 1. Standard curve for DNA-excess solution hybridization assay, using M13ß$_2$AR(111) template DNA.

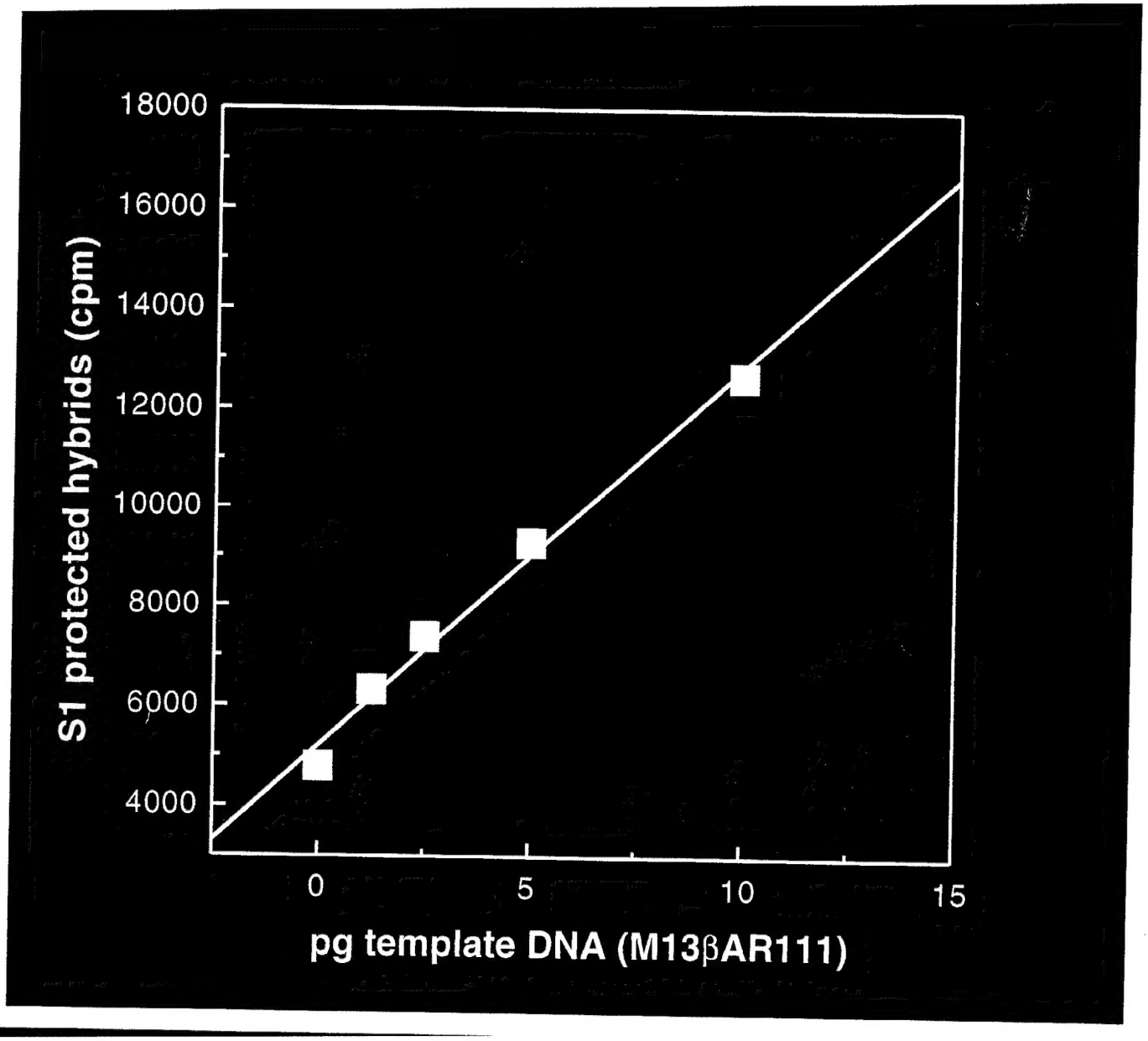


Figure 2. Saturation isoterms to asses the ßAR density and affinity in EBV-transformed human lymphocytes (Scatchard plot).
Both cytokines *decrease* the number of ß-adrenergic receptors without significantly changing the affinity. The corresponding B_{max} values: control, 12.6 fmol/mg protein; IL-1, 7.4 fmol/mg protein (59 % of control); IL-6, 9.3 fmol/mg protein (74 % of control). The affinity of the receptor is 60 pmol.

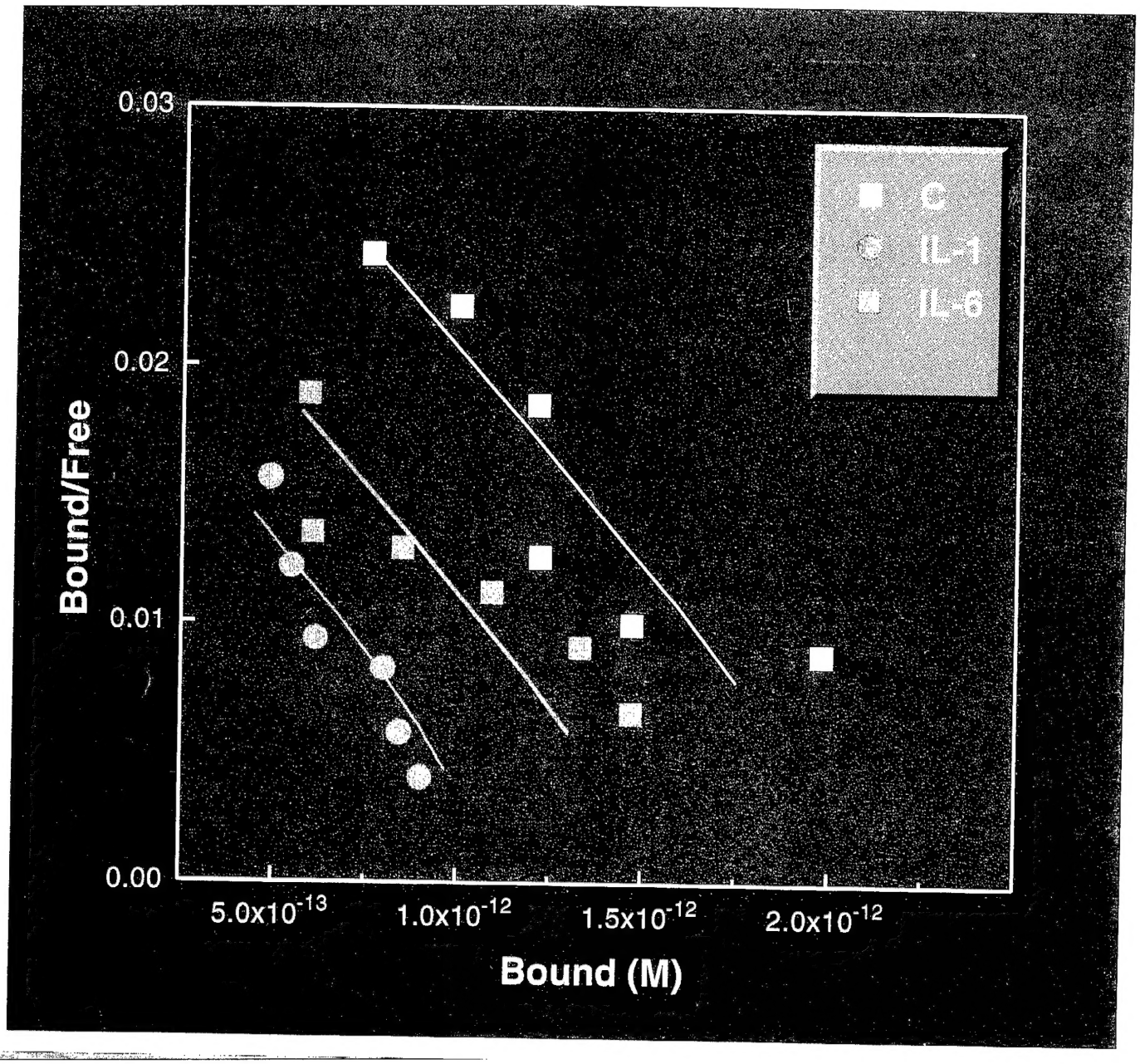


Figure 3. Saturation isoterms to asses the ßAR density and affinity in IM-9 lymphocytes (Scatchard plot).
Both cytokines *decrease* the number of ß-adrenergic receptors without significantly changing the affinity. The corresponding B_{max} values: control, 19.5 fmol/mg protein; IL-1, 15.3 fmol/mg protein (78 % of control); IL-6, 17.2 fmol/mg protein (88 % of control). The affinity of the receptor is 177 pmol.

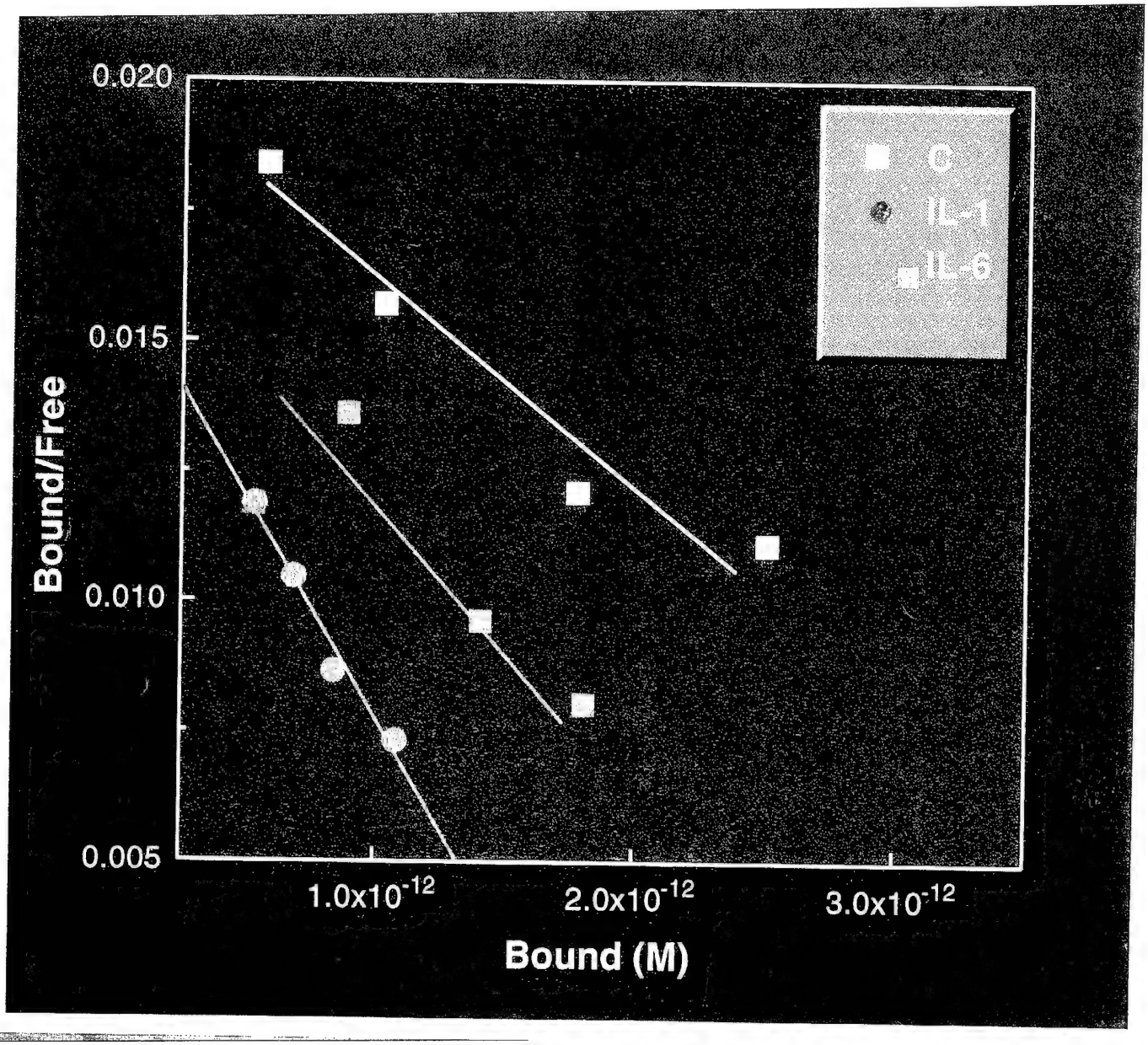


Figure 4. Isoproterenol-stimulated cAMP accumulation in EBV transformed lymphocytes after 24 hr IL-1 treatment (30 U/ml).
The IL-1 treated cells do not respond to the ß-adrenergic agonist stimulation.

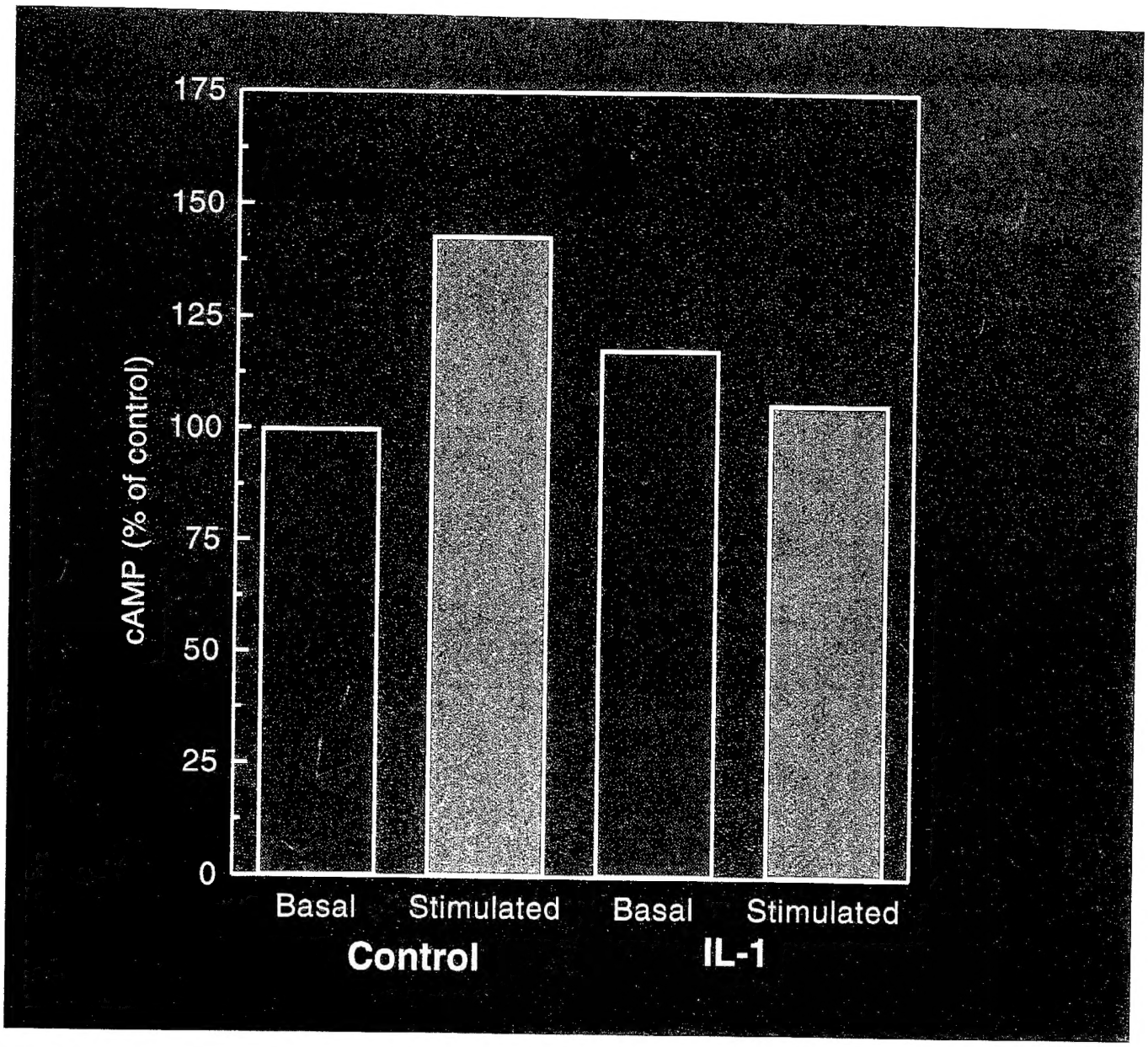


Figure 5. Steady state ß2AR mRNA concentrations in EBV transformed lymphocytes and in IM-9 lymphocytes following 24 hr IL-1 and IL-6 treatment (30 U/ml). Both cytokines *increase* the ß2AR message in both cell lines, but the effect is more pronounced in the EBV transformed cell line.

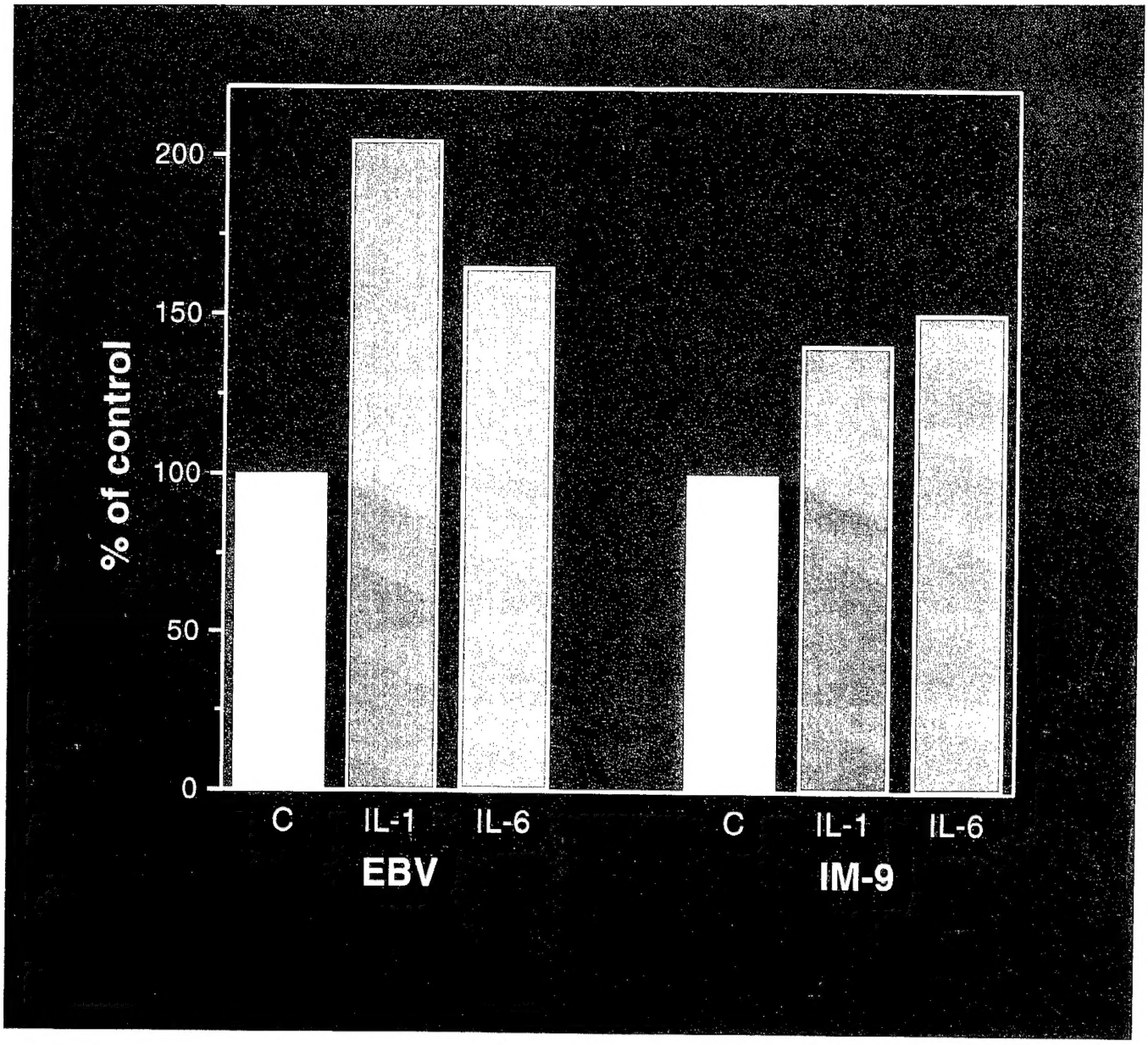
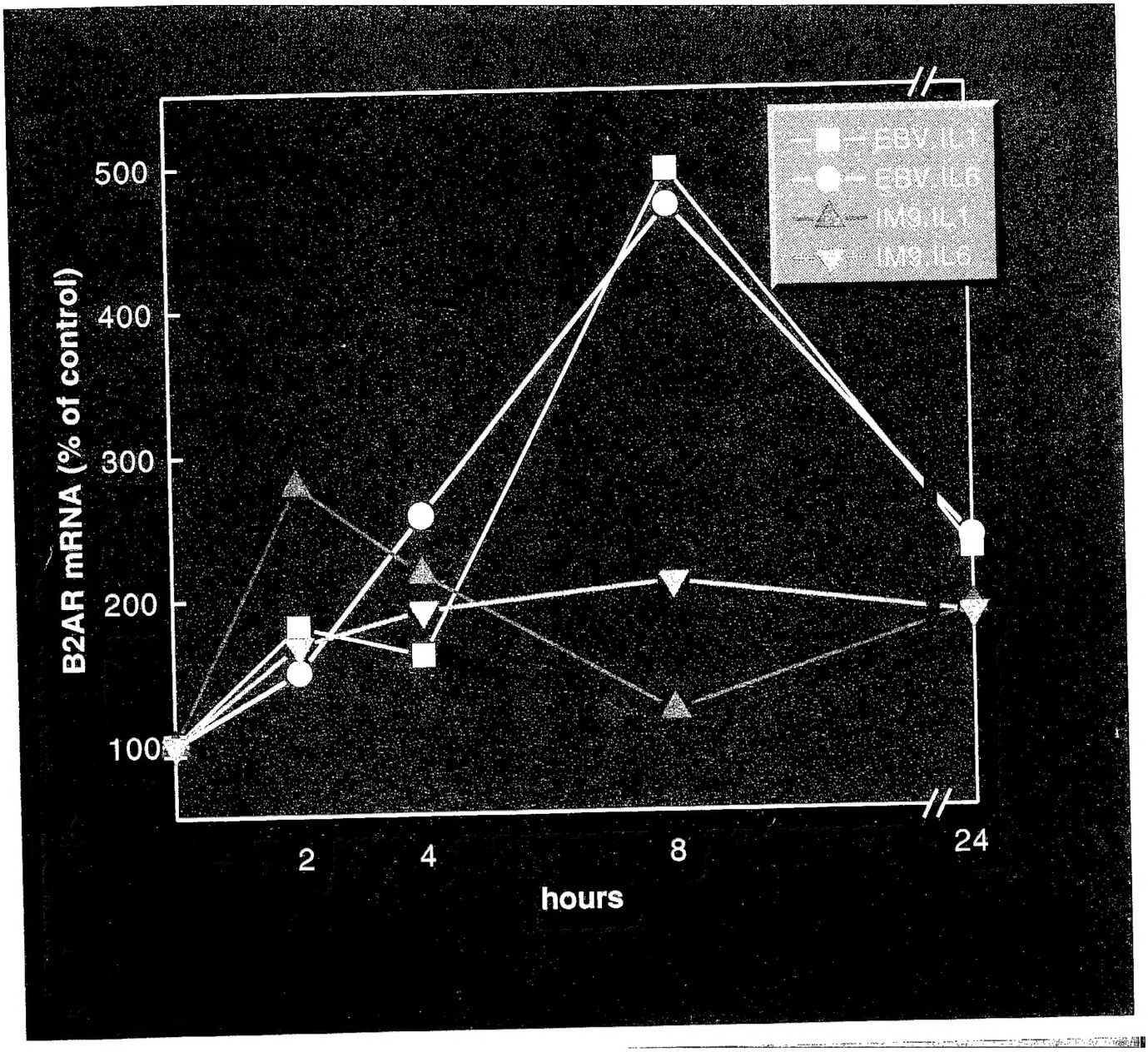


Figure 6. Time course of IL-1 and IL-6 effect on ß2AR mRNA levels in EBV-transformed and IM-9 cell lines.
That the cytokines had a maximum effect at 8 hr in EBV-transformed lymphocytes, and at 2 hr in IM-9 lymphocytes, and the effect on EBV cells was much more profound than that on the IM-9 cells.



Conclusions:

During the last year we succesfully completed the work proposed for the first phase. The results are being prepared for publication. Our results have clearly shown that the beta-2 adrenergic receptors (AR) are subject to regulation by elements of the immune system such as lymphokines. Therefore a clear link between the stress responisve elements and the immune system have been proven. These results indicate that stress response can be modulated by immune parameters.

We would like you to consider continuation of funding of this promising project so we may continue with the remaining phases of the project.

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